AN INTEGRIN HETERODIMER AND A SUBUNIT THEREOF

FIELD OF THE INVENTION

The present invention relates to a recombinant or isolated integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β, the subunit $\alpha 10$ thereof, homologues and fragments of said integrin and of said subunit $\alpha 10$ having similar biological activity, processes of producing the same, polynucleotides and oligonucleotides encoding the same, vectors and cells comprising the same, binding entities binding specifically to the same, and the use of the same.

BACKGROUND OF THE INVENTION

The integrins are a large family of transmembrane glycoproteins that mediate cell-cell and cell-matrix interactions (1-5). All known members of this superfamily are non-covalently associated heterodimers composed of an α- and a β-subunit. At present, 8 β-subunits ($\beta 1$-$\beta 8$) (6) and 16 α-subunits ($\alpha 1$-$\alpha 9$, αv, αM, αL, αX, αIIb, αE and αD) have been characterized (6-21), and these subunits associate to generate more than 20 different integrins. The $\beta 1$-subunit has been shown to associate with ten different α-subunits, $\alpha 1$-$\alpha 9$ and αv, and to mediate interactions with extracellular matrix proteins such as collagens, laminins and fibronectin. The major collagen binding integrins are $\alpha 1\beta 1$ and $\alpha 2\beta 1$ (22-25). The integrins $\alpha 3\beta 1$ and $\alpha 9\beta 1$ have also been reported to interact with collagen (26,27) although this interaction is not well understood (28). The extracellular N-terminal regions of the α and β integrin subunits are important in the binding of ligands (29,30). The N-terminal region of the α-subunits is composed of a seven-fold repeated sequence (12,31) containing FG and GAP consensus sequences. The repeats are predicted to fold into a β-propeller domain

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(32) with the last three or four repeats containing puta-
tive divalent cation binding sites. The α-integrin sub-
units α1, α2, αD, αE, αL, αM and αX contain a ~200 amino
acid inserted domain, the I-domain (A-domain), which

5   shows similarity to sequences in von Willebrand factor,
cartilage matrix protein and complement factors C2 and B
(33,34). The I-domain is localized between the second and
third FG-GAP repeats, it contains a metal ion-dependent
adhesion site (MIDAS) and it is involved in binding of

10   ligands (35-38).

Chondrocytes, the only type of cells in cartilage,
express a number of different integrins including α1β1,
α2β1, α3β1, α5β1, α6β1, αvβ3, and αvβ5 (39-41). It has
been shown that α1β1 and α2β1 mediate chondrocyte inter-

15   actions with collagen type II (25) which is one of the
major components in cartilage. It has also been shown
that α2β1 is a receptor for the cartilage matrix protein
chondroadherin (42).

20   SUMMARY OF THE INVENTION
The present invention relates to a novel collagen
type II binding integrin, comprising a subunit α10 in
association with a subunit β, especially subunit β1, but
also other β-subunits may be contemplated. In preferred

25   embodiments, this integrin has been isolated from human
or bovine articular chondrocytes, and human chondrosar-
coma cells.

The invention also encompasses integrin homologues
of said integrin, isolated from other species, such as

30   bovine integrin heterodimer comprising a subunit α10 in
association with a subunit β, preferably β1, as well as
homologues isolated from other types of human cells or
from cells originating from other species.

The present invention relates in particular to a

35   recombinant or isolated integrin subunit α10 comprising
the amino acid sequence shown in SEQ ID No. 1 or SEQ ID
No. 2, and homologues and or fragments thereof having the

same biological activity.

The invention further relates to a process of producing a recombinant integrin subunit α10 comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID

5   No. 2, or homologues or fragments thereof having similar biological activity, which process comprises the steps of

a) isolating a polynucleotide comprising a nucleotide sequence coding for a integrin subunit α10, or homologues or fragments thereof having similar biological

10 activity,

b) constructing an expression vector comprising the isolated polynucleotide,

c) transforming a host cell with said expression vector,

15 d) culturing said transformed host cell in a culture medium under conditions suitable for expression of integrin subunit α10, or homologues or fragments thereof having similar biological activity, in said transformed host cell, and, optionally,

20 e) isolating the integrin subunit α10, or homologues or fragments thereof having the same biological activity, from said transformed host cell or said culture medium.

The integrin subunit α10, or homologues or fragments thereof having the same biological activity, can also be

25 provided by isolation from a cell in which they are naturally present.

The invention also relates to an isolated polynucleotide comprising a nucleotide coding for a integrin subunit α10, or homologues or fragments thereof having

30 similar biological activity, which polynucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or parts thereof.

The invention further relates to an isolated polynucleotide or oligonucleotide which hybridises to a DNA

35 or RNA encoding an integrin subunit α10, having the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof, wherein said polyoligo-

nucleotide or oligonucleotide fails to hybridise to a DNA
or RNA encoding the integrin subunit α1.

The invention relates in a further aspect to vectors
comprising the above polynucleotides, and to cells con-
taining said vectors and cells that have polynucleotides
or oligonycleotides as shown in SEQ ID No. 1 or 2 inte-
grated in their genome.

The invention also relates to binding entities hav-
ing the capability of binding specifically to the inte-
grin subunit α10 or to homologues or fragments thereof,
such as proteins, peptides, carbohydrates, lipids, natu-
ral ligands, polyclonal antibodies or monoclonal anti-
bodies.

In a further aspect, the invention relates to a
recombinant or isolated integrin heterodimer comprising
a subunit α10 and a subunit β, in which the subunit α10
comprises the amino acid sequence shown in SEQ ID No. 1
or SEQ ID No. 2, or homologues or fragments thereof hav-
ing similar biological activity.

In a preferred embodiment thereof, the subunit β is
β1.

The invention also relates to a process of producing
a recombinant integrin heterodimer comprising a subunit
α10 and a subunit β, in which the subunit α10 comprises
the amino acid sequence shown in SEQ ID No. 1 or SEQ ID
No. 2, which process comprises the steps of

a) isolating one polynucleotide comprising a nucleo-
tide sequence coding for a subunit α10 of an integrin
heterodimer and, optionally, another polynucleotide com-
prising a nucleotide sequence coding for a subunit β of
an integrin heterodimer, or for homologues or fragments
thereof having similar biological activity,

b) constructing an expression vector comprising said
isolated polynucleotide coding for said subunit α10 in
combination with an expression vector comprising said
isolated nucleotide coding for said subunit β,

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c) transforming a host cell with said expression vectors,

d) culturing said transformed host cell in a culture medium under conditions suitable for expression of an integrin heterodimer comprising a subunit α10 and a subunit β, or homologues or fragments thereof similar biological activity, in said transformed host cell, and, optionally,

e) isolating the integrin heterodimer comprising a subunit α10 and a subunit β, or homologues or fragments thereof having the same biological activity, from said transformed host cell or said culture medium.

The integrin heterodimer, or homologues or fragments thereof having similar biological activity, can also be provided by isolation from a cell in which they are naturally present.

The invention further relates to a cell containing a first vector, said first vector comprising a polynucleotide coding for a subunit α10 of an integrin heterodimer, or for homologues or parts thereof having similar biological activity, which polynucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof, and, optionally, a second vector, said second vector comprising a polynucleotide coding for a subunit β of an integrin heterodimer, or for homologues or fragments thereof.

In still another aspect, the invention relates to binding entities having the capability of binding specifically to the integrin heterodimer comprising a subunit α10 and a subunit β, or to homologues or fragments thereof having similar biological activity, preferably wherein the subunit β is β1. Preferred binding entities are proteins, peptides, carbohydrates, lipids, natural ligands, polyclonal antibodies and monoclonal antibodies.

In a further aspect, the invention relates to a fragment of the integrin subunit α10, which fragment is a peptide chosen from the group comprising peptides of

the cytoplasmic domain, the I-domain and the spliced
domain.

In one embodiment, said fragment is a peptide com-
prising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

5   In another embodiment, said fragment comprises the
amino acid sequence from about amino acid no. 952 to
about amino acid no. 986 of SEQ ID No. 1.

In a further embodiment, said fragment comprises
the amino acid sequence from about amino acid No. 140

10   to about amino acid No. 337 in SEQ ID No. 1.

Another embodiment of the invention relates to a
polynucleotide or oligonucleotide coding for a fragment
of the human integrin subunit α10. In one embodiment this
polynucleotide of oligonucleotide codes for a fragment

15   which is a peptide chosen from the group comprising pep-
tides of the cytoplasmic domain, the I-domain and the
spliced domain. In further embodiments the polynucleotide
or oligonucleotide codes for the fragments defined above.

The invention also relates to binding entities hav-

20   ing the capability of binding specifically to a fragment
of the integrin subunit α10 as defined above.

The invention also relates to a process of using an
integrin subunit α10 comprising the amino acid sequence
shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin

25   heterodimer comprising said subunit α10 and a subunit β,
or a homologue or fragment of said integrin or subunit
having similar biological activity, as a marker or target
molecule of cells or tissues expressing said integrin
subunit α10, which cells or tissues are of animal includ-

30   ing human origin.

In an embodiment of this process the fragment is a
peptide chosen from the group comprising peptides of the
cytoplasmic domain, the I-domain and the spliced domain.

In further embodiments of said process the frag-

35   ment is a peptide comprising the amino acid sequence
KLGFFAHKKIPEEEKREEKLEQ, or a fragment comprising the
amino acid sequence from about amino acid No. 952 to

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about amino acid No. 986 of SEQ ID No. 1, or a fragment
comprising the amino acid sequence from about amino acid
no. 140 to about amino acid no. 337 of SEQ ID no. 1.

5   The subunit β is preferably β1. The cells are pre-
ferably chosen from the group comprising chondrocytes,
smooth muscle cells, endothelial cells, osteoblasts and
fibroblasts.

       Said process may be used during pathological condi-
tions involving said subunit α10, such as pathological
10   conditions comprising damage of cartilage, or comprising
trauma, rheumatoid arthritis and osteoarthritis.

       Said process may be used for detecting the formation
of cartilage during embryonal development, or for detect-
ing physiological or therapeutic reparation of cartilage.
15   Said process may also be used for selection and ana-
lysis, or for sorting, isolating or purification of chon-
drocytes.

       A further embodiment of said process is a process
for detecting regeneration of cartilage or chondrocytes
20   during transplantation of cartilage or chondrocytes.

       A still further embodiment of said process is a pro-
cess for in vitro studies of differentiation of chondro-
cytes.

       The invention also comprises a process of using
25   binding entities having the capability of binding speci-
fically to an integrin subunit α10 comprising the amino
acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or
an integrin heterodimer comprising said subunit α10 and a
subunit β, or to homologues or fragments thereof having
30   similar biological activity, as markers or target mole-
cules of cells or tissues expressing said integrin sub-
unit α10, which cells or tissues are of animal including
human origin.

       The fragment in said process may be a peptide chosen
35   from the group comprising peptides of the cytoplasmic
domain, the I-domain and the spliced domain. In prefer-
red embodiments said fragment is a peptide comprising the

amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or a fragment
comprising the amino acid sequence from about amino acid
No. 952 to about amino acid No. 986 of SEQ ID No. 1, or
a fragment comprising the amino acid sequence from about
amino acid No. 140 to about amino acid no. 337 of SEQ ID
No. 1.

The process may also be used for detecting the pre-
sence of an integrin subunit $\alpha 10$ comprising the amino
acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or
of an integrin heterodimer comprising said subunit $\alpha 10$
and a subunit β, or of homologues or fragments thereof
having similar biological activity.

In a further embodiment said process is a process
for determining the differentiation-state of cells during
embryonic development, angiogenesis, or development of
cancer.

In a still further embodiment this process is a
process for detecting the presence of an integrin sub-
unit $\alpha 10$, or of a homologue or fragment of said integrin
subunit having similar biological activity, on cells,
whereby a polynucleotide or oligonucleotide chosen from
the group comprising a polynucleotide or oligonucleotide
chosen from the nucelotide sequence shown in SEQ ID No. 1
is used as a marker under hybridisation conditions where-
in said polynucleotide or oligonucleotide fails to
hybridise to a DNA or RNA encoding an integrin subunit
$\alpha 1$. Said cells may be chosen from the group comprising
chondrocytes, smooth muscle cells, endothelial cells,
osteoblasts and fibroblasts. Said integrin fragment may
be a peptide chosen from the group comprising peptides
of the cytoplasmic domain, the I-domain and the spliced
domain, such as a peptide comprising the amino acid
sequence KLGFFAHKKIPEEEKREEKLEQ, or a fragment comprising
the amino acid sequence from about amino acid no. 952 to
about amino acid no. 986 of SEQ ID No. 1, or a fragment
comprising the amino acid sequence from about amino acid
No. 140 to about amino acid no. 337 of SEQ ID No. 1.

In a still further embodiment the process is a pro-
cess for determining the differentiation-state of cells
during development, in pathological conditions, in tissue
regeneration or in therapeutic and physiological repara-
5   tion of cartilage. The pathological conditions may be any
pathological conditions involving the integrin subunit
$\alpha 10$, such as rheumatoid arthritis, osteoarthrosis or
cancer. The cells may be chosen from the group compris-
ing chondrocytes, smooth muscle cells, endothelial cells,
10   osteoblasts and fibroblasts.

The invention also relates to a process for deter-
mining the differentiation-state of cells during develop-
ment, in pathological conditions, in tissue regeneration
and in therapeutic and physiological reparation of car-
15   tilage, whereby a polynucleotide or oligonucleotide
chosen from the nucleotide sequence shown in SEQ ID No. 1
is used as a marker under hybridisation conditions where-
in said polynucleotide or oligonucleotide fails to
hybridise to a DNA or RNA encoding an integrin subunit
20      $\alpha 1$. Embodiments of this aspect comprise a process, where-
by said polynucleotide or oligonucleotide is a poly-
nucleotide or oligonucleotide coding for a peptide
chosen from the group comprising peptides of the cyto-
plasmic domain, the I-domain and the spliced domain,
25   such as a polynucleotide or oligonucleotide coding
for a peptide comprising the amino acid sequence
KLGFFAHKKIPEEEKREEKLEQ, or comprising the amino acid
sequence from about amino acid No. 952 to about amino
acid no. 986 of SEQ ID No. 1, or the amino acid sequence
30   from about amino acid No. 140 to about amino acid No. 337
of SEQ ID No. 1. Said pathological conditions may be any
pathological conditions involving the integrin subunit
$\alpha 10$, such as rheumatoid arthritis, osteoarthrosis or
cancer, or atherosclerosis or inflammation. Said cells
35   may be chosen from the group comprising chondrocytes,
smooth muscle cells, endothelial cells, osteoblasts and
fibroblasts.

In a further aspect the invention relates to a phar-
maceutical composition comprising as an active ingredient
a pharmaceutical agent or an antibody which is capable of
using an integrin heterodimer comprising a subunit α10
5   and a subunit β, or the subunit α10 thereof, or a homo-
logue or fragment of said integrin or subunit α10 having
similar biological activity, as a target molecule. An
embodiment of said pharmaceutical composition is intended
for use in stimulating, inhibiting or blocking the forma-
10 tion of cartilage, bone or blood vessels. A further embo-
diment comprises a pharmaceutical composition for use in
preventing adhesion between tendon/ligaments and the
surrounding tissue after infection, inflammation and
after surgical intervention where adhesion impairs the
15 function of the tissue.

The invention is also related to a vaccine compris-
ing as an active ingredient an integrin heterodimer com-
prising a subunit α10 and a subunit β, or the subunit α10
thereof, or a homologue or fragment of said integrin or
20 subunit α10, or DNA or RNA coding for said integrin sub-
unit α10.

A further aspect of the invention is related to the
use of the integrin subunit α10 as defined above as a
marker or target in transplantation of cartilage or chon-
25 drocytes.

A still further aspect of the invention is related
to a method of using binding entities having the capabi-
lity of binding specifically to an integrin subunit α10
comprising the amino acid sequence shown in SEQ ID No. 1
30 or SEQ ID No. 2, or an integrin heterodimer comprising
said subunit α10 and a subunit β, or to homologues or
fragments thereof having similar biological activity, for
promoting adhesion of chondrocytes and/or osteoblasts to
surfaces of implants to stimulate osseointegration.

35   The invention is also related to the use of an inte-
grin subunit α10 or an integrin heterodimer comprising
said subunit α10 and a subunit β as a target for anti-

adhesive drugs or molecules in tendon, ligament, skeletal
muscle or other tissues where adhesion impairs the func-
tion of the tissue.

The invention also relates to a method of stimulat-
ing, inhibiting or blocking the formation of cartilage or
bone, comprising administration to a subject a suitable
amount of a pharmaceutical agent or an antibody which is
capable of using an integrin heterodimer comprising a
subunit α10 and a subunit β, or the subunit α10 thereof,
or a homologue or fragment of said integrin or subunit
α10 having similar biological activity, as a target mole-
cule.

In another embodiment the invention is related to a
method of preventing adhesion between tendon/ligaments
and the surrounding tissue after infection, inflammation
and after surgical intervention where adhesion impairs
the function of the tissue, comprising administration to
a subject a suitable amount of a pharmaceutical agent or
an antibody which is capable of using a integrin hetero-
dimer comprising a subunit α10 and a subunit β, or the
subunit α10 thereof, or a homologue or fragment of said
integrin or subunit α10 having similar biological acti-
vity, as a target molecule.

The invention also relates to a method of stimulat-
ing extracellular matrix synthesis and repair by activa-
tion or blockage of an integrin heterodimer comprising
a subunit α10 and a subunit β, or of the subunit α10
thereof, or of a homologue or fragment of said integrin
or subunit α10 having similar biological activity.

In a further aspect the invention relates to a
method of in vitro detecting the presence of integrin
binding entities, comprising interaction of an integrin
heterodimer comprising a subunit α10 and a subunit β, or
the subunit α10 thereof, or a homologue or fragment of
said integrin or subunit, with a sample, thereby causing
said integrin, subunit α10, or homologue or fragment
thereof having similar biological activity, to modulate

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the binding to its natural ligand or other integrin bind-
ing proteins present in said sample.

    The invention also relates to a method of in vitro
studying consequences of the interaction of a human
5   heterodimer integrin comprising a subunit α10 and a sub-
unit β, or the subunit α10 thereof, or a homologue or
fragment of said integrin or subunit, with an integrin
binding entity and thereby initiate a cellular reaction.
Said consequences may be measured as alterations in cel-
10   lular functions.

    A still further aspect of the inventions relates to
a method of using DNA or RNA encoding an integrin subunit
α10 or homologues or fragments thereof as a molecular
target. In an embodiment of this aspect, a polynucleotide
15   or oligonucleotide hybridises to the DNA or RNA encoding
an integrin subunit α10 or homologues or fragments there-
of, whereby said polynucleotide or oligonucleotide fails
to hybridise to a DNA or RNA encoding en integrin subunit
α1.

20   The invention also relates to a method of using a
human heterodimer integrin comprising a subunit α10 and
a subunit β, or the subunit α10 thereof, or a homologue
or fragment of said integrin or subunit, or a DNA or RNA
encoding an integrin subunit α10 or homologues or frag-
25   ments thereof, as a marker or target molecule during
angiogenesis.

BRIEF DESCRIPTION OF THE FIGURES

    Fig.1 Affinity purification of the α10 integrin sub-
unit on collagen type II-Sepharose.

30   Fig. 2. Amino acid sequences of peptides from the
bovine α10 integrin subunit.

    Fig. 3a. Affinitypurification and immunoprecipi-
tation of the integrin subunit α10 from bovine chondro-
cytes.

35   Fig. 3b. Affinitypurification and immunoprecipita-
tion of the integrin subunit α10 from human chondrocytes.

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Fig. 3c. Affinitypurification and immunoprecipita-
tion of the integrin subunit α10 from human chondrosar-
coma cells.

Fig. 4. A 900 bp PCR-fragment corresponding to the
bovine integrin subunit α10

Fig. 5. Schematic map of the three overlapping α10
clones.

Fig. 6. Nucleotide sequence and deduced amino acid
sequence of the human α10 integrin subunit.

Fig. 7. Northern blot of integrin α10 mRNA.

Fig. 8 Immunoprecipitation of the α10 integrin sub-
unit from human chondrocytes using antibodies against the
cytoplasmic domain of α10 (a). Western blot of the α10
associated β-chain (b).

Fig. 9. Immunostaining of α10 integrin in human
articular cartilage.

Fig. 10 Immunostaining of α10 integrin in 3 day
mouse limb cartilage.

Fig 11. Immunostaining of α10 integrin in 13.5 day
mouse embryo.

Fig 12. Hybridisation of α10 mRNA in various human
tissues.

Fig. 13 Immunostaining of fascia around tendon (a),
skeletal muscle (b) and heart valves (c) in 3 day mouse
limb.

Fig. 14. PCR fragments corresponding to α10 integrin
subunit from human chondrocytes, human endothelial cells,
human fibroblasts and rat tendon.

Fig 15. Partial genomic nucleotide sequence of the
human integrin subunit α10.

Fig 16. Upregulation of α10 integrin subunit in
chondrocytes cultured in alginate.

Fig 17. Immunoprecipitation of the α10 integrin sub-
unit from human smooth muscle cells

DETAILED DESCRIPTION OF THE INVENTION

The present invention demonstrate that human and

bovine chondrocytes express a novel, collagen type
II-binding integrin in the β1-family. An earlier study
presented some evidence for that human chondrosarcoma
cells also express this integrin (25). Immunoprecipita-
5   tion experiments using antibodies against the integrin
subunit β1 revealed that this novel α-integrin subunit
had an apparent molecular weight (M_r) of approximately
160 kDa under reducing conditions, and was slightly
larger than the α2 integrin subunit. To isolate this
10   α-subunit collagen type II-binding proteins were affinity
purified from bovine chondrocytes. The chondrocyte lysate
was first applied to a fibronectin-Sepharose precolumn
and the flow through was then applied to a collagen type
II-Sepharose column. A protein with M_r of approximately
15   160 kD was specifically eluted with EDTA from the colla-
gen column but not from the fibronectin column. The M_r of
this protein corresponded with the M_r of the unidentified
β1-related integrin subunit. The 160 kD protein band was
excised from the SDS-PAGE gel, digested with trypsin and
20   the amino acid sequences of the isolated peptides were
analysed.

     Primers corresponding to isolated peptides amplified
a 900 bp PCR-fragment from bovine cDNA which was cloned,
sequenced and used for screening of a human articular
25   chondrocyte λZapII cDNA library to obtain the human inte-
grin α-subunit homologue. Two overlapping clones, hc1 and
hc2 were isolated, subcloned and sequenced. These clones
contained 2/3 of the nucleotide sequence including the 3'
end of the cDNA. A third clone which contained the 5'end
30   of the α10 cDNA, was obtained using the RACE technique.
Sequence analysis of the 160 kD protein sequence showed
that it was a member of the integrin α-subunit family and
the protein was named α10.

     The deduced amino acid sequence of α10 was found to
35   share the general structure of the integrin α-subunits
described in previously published reports (6-21). The
large extracellular N-terminal part of α10 contains a

seven-fold repeated sequence which was recently predicted
to fold into a β-propeller domain (32). The integrin sub-
unit α10 contains three putative divalent cation-binding
sites (DxD/NxD/NxxxD) (53), a single spanning transmem-
brane domain and a short cytoplasmic domain. In contrast
to most α-integrin subunits the cytoplasmic domain of α10
does not contain the conserved sequence KxGFF (R/K) R.
The predicted amino acid sequence in α10 is KLGFFAH.
Several reports indicate that the integrin cytoplasmic
domains are crucial in signal transduction (54) and that
membrane-proximal regions of both α- and β-integrin cyto-
plasmic domains are involved in modulating conformation
and affinity state of integrins (55-57). It is suggested
that the GFFKR motif in α-chains are important for asso-
ciation of integrin subunits and for transport of the
integrin to the plasma membrane (58). The KxGFFKR domain
has been shown to interact with the intracellular protein
calreticulin (59) and interestingly, calreticulin-null
embryonic stem cells are deficient in integrin-mediated
cell adhesion (60). It is therefor possible that the
sequence KLGFFAH in α10 have a key function in regulating
the affinity between α10β1 and matrix proteins.

Integrin α subunits are known to share an overall
identity of 20-40% (61). Sequence analysis showed that
the α10 subunit is most closely related to the I-domain
containing α-subunits with the highest identity to α1
(37%) and α2 (35%). The integrins α1β1 and α2β1 are known
receptors for both collagens and laminins (24;62;63) and
we have also recently demonstrated that α2β1 interacts
with the cartilage matrix protein chondroadherin (42).
Since α10β1 was isolated on a collagen type II-Sepharose,
we know that collagen type II is a ligand for α10β1. We
have also shown by affinity purification experiments that
α10β1 interacts with collagen type I but it remains to be
seen whether laminin or chondroadherin are also ligands
for this integrin.

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The α10 associated β-chain migrated as the β1 integrin subunit both under reducing and non-reducing conditions. To verify that the α10 associated β-chain indeed is β1, chondrocyte lysates were immunoprecipitated with
5   antibodies against α10 or β1 followed by Western blot using antibodies against the β1-subunit. These results clearly demonstrated that α10 is a member of the β1-integrin family. However, the possibility that α10 combine also with other β-chains can not be excluded..

10   A polyclonal peptide antibody raised against the cytoplasmic domain of α10 precipitated two protein bands with M_r of approximately 160 kD (α10) and 125 kD (β1) under reducing conditions. Immunohistochemistry using the α10-antibody showed staining of the chondrocytes in tis-
15   sue sections of human articular cartilage. The antibody staining was clearly specific since preincubation of the antibody with the α10-peptide completely abolished the staining. Immunohistochemical staining of mouse limb sections from embryonic tissue demonstrated that α10 is
20   upregulated during condensation of the mesenchyme. This indicate that the integrin subunit α10 is important during the formation of cartilage. In 3 day old mice α10 was found to be the dominating collagen binding integrin subunit which point to that α10 has a key function in
25   maintaining normal cartilage functions.

Expression studies on the protein and mRNA level show that the distribution of α10 is rather restrictive. Immunohistochemistry analyses have shown that α10 integrin subunit is mainly expressed in cartilage but it is
30   also found in perichondrium, periosteum, ossification groove of Ranvier, in fascia surrounding tendon and skeletal muscle and in the tendon-like structures in the heart valves. This distribution point to that α10 integrin subunit is present also on fibroblasts and
35   osteoblasts. PCR amplification of cDNA from different cell types revealed the presence of an alternatively spliced α10 integrin subunit. This spliced α10 was domi-

nating in fibroblasts which suggests that α10 in fibro-
blasts may have a different function compared to α10 pre-
sent on chondrocytes.

Expression of the integrin subunit α10 was found to
decrease when chondrocytes were cultured in monolayer. In
contrast, the expression of α10 was found to increase
when the cells were cultured in alginate beads. Since the
latter culturing model is known to preserve the phenotype
of chondrocytes the results suggest that α10 can function
as marker for a differentiated chondrocyte.

Adhesion between tendon/ligaments and the surround-
ing tissue is a well-known problem after infection,
injury and after surgical intervention. Adhesion between
tendon and tendon sheets impairs the gliding function and
cause considerable problems especially during healing of
tendons in e.g. the hand and fingers leading to func-
tional incapacity. The localisation of the α10 integrin
subunit in the fascia of tendon and skeletal muscle makes
α10 a possible target for drugs and molecules with anti-
adhesive properties that could prevent impairment of the
function of tendon/ligament. The integrin subunit α10 can
also be a target for drugs or molecules with anti-adhe-
sive properties in other tissues where adhesion is a
problem.

EXAMPLES

Example 1
        Affinity purification of the α10integrin subunit on
collagen type II-Sepharose.
Materials and Methods
        Bovine chondrocytes, human chondrocytes or human
chondrosarcoma cells were isolated as described earlier
[Holmvall et al, Exp Cell Res, 221, 496-503 (1995),
Camper et al, JBC, 273, 20383-20389 (1998)]. A Triton
X-100 lysate of bovine chondrocytes was applied to a
fibronectin-Sepharose precolumn followed by a collagen

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type II-Sepharose column and the integrin subunit α10 was
eluted from the collagen type II-column by EDTA (Camper
et al, JBC, 273, 20383-20389 (1998). The eluted proteins
were precipitated by methanol/chloroform, separated by
5   SDS-PAGE under reducing conditions and stained with
Coomassie blue. (Camper et al, JBC, 273, 20383-20389
(1998). Peptides from the α10 protein band were isolated
by in-gel digestion with a trypsin and phase liquid chro-
matography and sequenced by Edman degradation (Camper et
10   al, JBC, 273, 20383-20389 (1998).
Results

     Fig 1 shows EDTA-eluted proteins from the fibronec-
tin-Sepharose (A), flow-through from the collagen type
II-Sepharose column (B) and EDTA-eluted proteins from the
15   collagen type II-Sepharose (C). The α10 integrin subunit
(160 kDa) which was specifically eluted from the collagen
type II column is indicated with an arrow. Figure 2 shows
the amino acid sequences of six peptides that were iso-
lated from the bovine integrin subunit α10. Figures 3 a,
20   b, and c show that the α10 integrin subunit is present
on bovine chondrocytes (3a), human chondrocytes (3b) and
human chondrosarcoma cells (3c). The affinity for colla-
gen type II, the coprecipitation with β1-integrin subunit
and the molecular weight of 160 kDa under reducing condi-
25   tions identify the α10 integrin subunit on the different
cells. These results show that α10 can be isolated from
chondrocytes and from chondrosarcoma cells.

Example 2
30   Amplification of PCR fragment corresponding to
bovine α10 integrin subunit.
Materials and methods
     The degenerate primers GAY AAY ACI GCI CAR AC
(DNTAQT, forward) and TIA TIS WRT GRT GIG GYT (EPHHSI,
35   reverse) were used in PCR (Camper et al, JBC, 273, 20383-
20389 (1998) to amplify the nucleotide sequence corre-
sponding to the bovine peptide 1 (Figure 2). A 900 bp

PCR-fragment was then amplified from bovine cDNA using an internal specific primer TCA GCC TAC ATT CAG TAT (SAYIQY, forward) corresponding to the cloned nucleotide sequence of peptide 1 together with the degenerate primer ICK RTC

5   CCA RTG ICC IGG (PGHWDR, reverse) corresponding to the bovine peptide 2 (Figure2). Mixed bases were used in positions that were twofold degenerate and inosines were used in positions that are three- or fourfold degenerate. mRNA isolation and cDNA synthesis was done as earlier

10 described (Camper et al, JBC, 273, 20383-20389 (1998)). The purified fragment was cloned, purified and sequenced as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)).

Results

15   The nucleotide sequence of peptide 1 (Figure 2) was obtained by PCR-amplification, cloning and sequencing of bovine cDNA. From this nucleotide sequence an exact primer was designed and applied in PCR-amplification with degenerate primers corresponding to peptides

20 2-6 (Figure 2). Primers corresponding to peptides 1 and 2 amplified a 900 bp PCR-fragment from bovine cDNA (Figure 4).

Example 3

25   Cloning and sequence analysis of the human α10 integrin subunit

Material and methods

        The cloned 900bp PCR-fragment, corresponding to bovine α10-integrin, was digoxigenin-labelled according

30 to the DIG DNA labelling kit (Boehringer Mannheim) and used as a probe for screening of a human articular chondrocyte λZapII cDNA library (provided by Michael Bayliss, The Royal Veterinary Basic Sciences, London, UK)(52). Positive clones containing the pBluescript SK+ plasmid

35 with the cDNA insert were rescued from the ZAP vector by in vivo excision as described in the ZAP-cDNA® synthesis kit (Stratagene). Selected plasmids were purified and

sequenced as described earlier (Camper et al, JBC, 273,
20383-20389 (1998)) using T3, T7 and internal specific
primers. To obtain cDNA that encoded the 5' end of α10 we
designed the primer AAC TCG TCT TCC AGT GCC ATT CGT GGG
5   (reverse; residue 1254-1280 in α10 cDNA) and used it for
rapid amplification of the cDNA 5' end (RACE) as describ-
ed in the Marathon™ cDNA Amplification kit (Clontech
INC., Palo Alto, CA).
Results
10   Two overlapping clones, hc1 and hc2 (Figure 5), were
isolated, subcloned and sequenced. These clones contained
2/3 of the nucleotide sequence including the 3' end of
the cDNA. A third clone (race1; Figure 5), which contain-
ed the 5'end of the α10 cDNA, was obtained using the
15   RACE technique. From these three overlapping clones of
α10 cDNA, 3884 nucleotides were sequenced. The nucleotide
sequence and deduced amino acid sequence is shown in
Figure 6. The sequence contains a 3504-nucleotide open
reading frame that is predicted to encode a 1167 amino
20   acid mature protein. The signal peptide cleavage site is
marked with an arrow, human homologues to bovine peptide
sequences are underlined and the I-domain is boxed. Metal
ion binding sites are indicated with a broken underline,
potential N-glycosylation sites are indicated by an
25   asterisk and the putative transmembrane domain is double
underlined. The normally conserved cytoplasmic sequence
is indicated by a dot and dashed broken underline.
      Sequence analysis demonstrate that α10 is a member
of the integrin α-subunit family.
30

Example 4
      Identification of a clone containing a splice
variant of α10
      One clone which was isolated from the human chon-
35   drocyte library (see Example 3) contained a sequence
that was identical to the sequence of α10 integrin sub-
unit except that the nucleotides between nt positions

2942 and 3055 were deleted. The splice variant of α10
was verified in PCR experiment using primers flanking
the splice region (see figure 14).

5   Example 5
       Identification of α10 integrin subunit by Northern
blot
Material and methods
       Bovine chondrocyte mRNA was purified using a
10   QuickPrep®Micro mRNA Purification Kit (Pharmacia Biotech,
Uppsala, Sweden), separated on a 1% agarose-formaldehyde
gel, transferred to nylon membranes and immobilised by UV
crosslinking. cDNA-probes were 32P-labelled with Random
Primed DNA Labeling Kit (Boehringer Mannheim). Filters
15   were prehybridised for 2-4 hours at 42°C in 5x SSE,
5x Denharts solution, 0.1 % SDS, 50 µg/ml· salmon sperm
DNA and 50% formamide and then hybridised over night
at 42 °C with the same solution containing the specific
probe (0.5-1 x 106 cpm/ml). Specifically bound cDNA-
20   probes were analysed using the phosphoimager system
(Fuji). Filters were stripped by washing in 0.1% SDS,
for 1 hour at 80°C prior to re-probing. The α10-integrin
cDNA-probe was isolated from the race1-containing plasmid
using the restriction enzymes BamHI (GIBCO BRL) and NcoI
25   (Boehringer Mannheim). The rat β1-integrin cDNA probe was
a kind gift from Staffan Johansson, Uppsala, Sweden.
Results
       Northern blot analysis of mRNA from bovine chondro-
cytes showed that a human α10 cDNA-probe hybridised with
30   a single mRNA of approximately 5.4 kb (Figure 7). As a
comparison, a cDNA-probe corresponding to the integrin
subunit α1 was used. This cDNA-probe hybridised a mRNA-
band of approximately 3.5 kb on the same filter. These
results show that a cDNA-probe against α10 can be used to
35   identify the α10 integrin subunit on the mRNA level.

22

Example 6

Preparation of antibodies against the integrin sub-
unit α10

A peptide corresponding to part of the α10 cytoplas-
5   mic domain, Ckkipeeekreekle (see figure 6) was synthesis-
ed and conjugated to keyhole limpet hemocyanin (KLH).
Rabbits were immunised with the peptide-KLH conjugate
to generate antiserum against the integrin subunit α10.
Antibodies recognising α10 were affinity purified on an
10   peptide-coupled column (Innovagen AB).

Example 7

Immunoprecipitation of the integrin subunit α10 from
chondrocytes
15   Material and methods

Human chondrocytes were ^{125}I-labelled, lyzed with
Triton X-100 and immunoprecipitated as earlier described
(Holmvall et al, Exp Cell Res, 221, 496-503 (1995),
Camper et al, JBC, 273, 20383-20389 (1998)). Triton X-100
20   lysates of 125I-labeled human chondrocytes were immuno-
precipitated with polyclonal antibodies against the inte-
grin subunits β1, α1, α2, α3 or α10. The immunoprecipi-
tated proteins were separated by SDS-PAGE (4-12%) under
non-reducing conditions and visualised using a phospho-
25   imager. Triton X-100 lysates of human chondrocytes immu-
noprecipitated with α10 or β1 were separated by SDS-PAGE
(8%) under non-reducing conditions and analysed by
Western blot using the polyclonal β1 antibody and chemi-
luminescent detection as described in Camper et al, JBC,
30   273, 20383-20389 (1998).
Results

The polyclonal peptide antibody, raised against the
cytoplasmic domain of α10, precipitated two protein bands
with Mr of approximately 160 kD (α10) and 125 kD (β1)
35   under reducing conditions. The α10 associated β-chain
migrated as the β1 integrin subunit (Figure 8a). To
verify that the α10 associated β-chain in chondrocytes

indeed is β1, chondrocyte lysates were immunoprecipitated
with antibodies against α10 orb β1 followed by Western
blot using antibodies against the β1-subunit (Figure 8b).
These results clearly demonstrated that α10 is a member

5   of the β1-integrin family. However, the results do not
exclude the possibility that α10 can associate with other
β-chains in other situations.

Example 8

10   Immunohistochemical staining of the integrin subunit
α10 in human and mouse cartilage
Material and methods
        Frozen sections of adult cartilage (trochlear
groove) obtained during surgery (provided by Anders

15 Lindahl, Salgrenska Hospital, Gothenburg, Sweden and
frozen sections from of 3 day old mouse limb were fixed
and prepared for immunohistochemistry as earlier describ-
ed (Camper et al, JBC, 273, 20383-20389 (1998)). Expres-
sion of α10 integrin subunit was analysed using the poly-

20 clonal antibody against the cytoplasmic domain as a pri-
mary antibody (see Example 6) and a secondary antibody
conjugated to peroxidase.
Results
        Figures 9 show immunostaining of human adult articu-

25 lar cartilage.
        The α10-antibody recognising the cytoplasmic domain
of α10 stained the chondrocytes in tissue sections of
human articular cartilage (A). The staining was depleted
when the antibody was preincubated with the α10- peptide

30 (B). A control antibody recognising the α9 integrin sub-
unit did not bind to the chondrocyte (C).
        Figures 10 shows that the α10 antibody stain the
majority of chondrocytes in the growing bone anlage (a
and b). The α10 antibody also recognised cells in the

35 ossification groove of Ranvier (b), especially the
osteoblast in the bone bark which are lining the carti-
lage in the metaphys are highly positive for α10. The

24

cells in the ossification groove of Ranvier are believed
to be important for the growth in diameter of the bone.
The integrin subunit α10 is also highly expressed in
perichondrium and periosteum. Cell in these tissues are
5   likely important in the repair of the cartilage tissue.
The described localisation of the integrin subunit α10
suggest that this integrin is important for the function
of the cartilage tissue.

10   Example 9
        Immunohistochemical staining of the integrin subunit
α10 during mouse development
Material and methods
        Frozen sections from mouse embryos (13.5 days) were
15   investigated for expression of α10 by immunhistochemi-
stry as described in Camper et al, JBC, 273, 20383-20389
(1998). Expression of α10 integrin subunit was analysed
using the polyclonal antibody against the cytoplasmic
domain as a primary antibody (see Example 6) and a secon-
20   dary antibody conjugated to peroxidase. The embryo sec-
tions were also investigated for expression of integrin
subunit α1 (monoclonal antibody from Pharmingen) and
collagen type II (monoclonal antibody, kind gift from
Dr John Mo, Lund University, Sweden).
25   Results
        Figure 11 show that α10 integrin subunit is unregu-
lated in the limb when the mesenchymal cells undergo con-
densation to form cartilage (a). Especially the edge of
the newly formed cartilage has high expression of α10.
30   The formation of cartilage is verified by the high
expression of the cartilage specific collage type II (b).
The control antibody against α1 integrin subunit showed
only weak expression on the cartilage (c). In other expe-
riments expression of α10 was found in all cartilage con-
35   taining tissues in the 3 day old mouse including limbs,
ribs and vertebrae. The upregulation of α10 during forma-
tion of cartilage suggest that this integrin subunit is

25

important both in the development of cartilage and bone
and in the repair of damaged cartilage tissue.

Example 10
5   mRNA expression of α10 in tissues other than articu-
lar cartilage
Material and methods
        Expression of α10 integrin subunit was examined on
the mRNA level in different human tissues. A Northern dot
10   blot with immobilised mRNA from the listed tissues in
Figure 12 was hybridised with an α10 integrin cDNA probe
isolated from the race 1-containing plasmid using the
restriction enzymes BamH1 and Nco1. The degree of hybrid-
isation was analysed using a phospho imager. The follow-
15   ing symbols denote mRNA level in increasing order: -, +,
++, +++, ++++.
Results
        Analysis of the hybridised mRNA showed that α10
was expressed in aorta, trachea, spinal cord, heart,
20   lung, and kidney (Figure 12). All other tissues appear-
ed negative for α10 expression. These results point to
a restricted distribution of the α10 integrin subunit.

Example 11
25   Immunohistochemical staining of α10 in fascia around
tendon and skeletal muscle and in tendon structures in
heart valves.
Materials and methods
        Frozen sections of adult cartilage (trochlear
30   groove) obtained during surgery (provided by Anders
Lindahl, Salgrenska Hospital, Gothenburg, Sweden and
frozen sections from of 3 day old mouse limb were fixed
and prepared for immunohistochemistry as earlier describ-
ed (Camper et al, JBC, 273, 20383-20389 (1998)). Expres-
35   sion of α10 integrin subunit was analysed using the poly-
clonal antibody against the cytoplasmic domain as a pri-

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mary antibody (see Example 6) and a secondary antibody
conjugated to peroxidase.
Results

As shown in figures 13 expression of α10 was found
5   in the fascia surrounding tendon (a) and skeletal muscle
(b) and in the tendon structures in the heart valves (c).
This localisation suggest that α10 can bind to other
matrix molecules in addition to the cartilage specific
collagen type II. The localisation of the integrin α10 on
10 the surface of tendons indicate that α10 can be involved
in unwanted adhesion that often occurs between tendon/
ligaments and the surrounding tissue after infection,
injury or after surgery.

15 Example 12
mRNA expression of α10 integrin subuhit in chondro-
cytes, endothelial cells and fibroblasts.
Material and methods
Isolation of mRNA, synthesis of cDNA and PCR ampli-
20 fication was done as earlier described (Camper et al,
JBC, 273, 20383-20389 (1998)).
Results
Figure 14 shows PCR amplification of α10 cDNA from
human articular chondrocytes (lanes A6 and B1), human
25 umbilical vein endothelial cells (lane A2), human fibro-
blasts (lane A4) and rat tendon (Fig 14b, lane B2). Lanes
1, 3, and 5 in figure 14 A show amplified fragments cor-
responding to the integrin subunit α2 in endothelial
cells, fibroblasts and chondrocytes, respectively. cDNA-
30 primers corresponding to the α10 sequence positions nt
2919-2943 (forward) and nt 3554-3578 (reverse) (see
Figure 6) were used to amplify α10 cDNA from the diffe-
rent cells. The figure shows that α10 was amplified in
all three cell types. Two fragments of α10 was amplified
35 which represent the intact form of α10 (larger fragment)
and a splice variant (smaller fragment). The larger frag-

ment was dominating in chondrocytes while the smaller
fragment was more pronounced in tendon (B2).

Example 13
        Construction of α10 mammalian expression vector.
        The full length protein coding sequence of α10 (com-
bined from 3 clones, see figure 6) was inserted into the
mammalian expression vector, pcDNA3.1/Zeo (Invitrogen).
The vector contains SV40 promoter and Zeosin selection
sequence. The α10 containing expression vector was trans-
fected into cells that express the β1-integrin subunit
but lack expression of the α10 subunit. Expression of the
α10 integrin subunit on the cell surface can be analysed
by immunoprecipitation and/or flow cytometry using anti-
bodies specific for α10. The ligand binding capacity and
the function of the inserted α10 integrin' subunit can be
demonstrated in cell adhesion experiment and in
signalling experiments.

Example 14
        Construction of mammalian expression vector contain-
ing a splice variant of α10.
        The full length protein coding sequence of the
splice variant of α10 (nt 2942-nt3055 deleted) was
inserted into the mammalian expression vector pcDNA3
(see Example 13). Expression and function of the splice
variant can be analysed as described in example 13 and
compared with the intact α10 integrin subunit.

Example 15
        Partial isolation and characterisation of the α10
integrin genomic DNA
Material and methods
        Human α10 cDNA, isolated from the race1-containing
plasmid using the restriction enzymes BamHI (GIBCO BRL)
and NcoI (Boehringer Mannheim), was ^{32}P-labelled and used
as a probe for screening of a mouse 129 cosmid library

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(provided by Reinhard Fässler, Lund University). Positive
clones were isolated and subcloned. Selected plasmids
were purified and sequenced as described earlier (Camper
et al, JBC, 273, 20383-20389 (1998)) using T3, T7 and
5   internal specific primers. Primers corresponding to mouse
genomic DNA were then constructed and used in PCR to
amplify and identify the genomic sequence of α10 from the
cosmid clones.
Results
10   Figure 15 shows 7958 nt of the α10 gene. This par-
tial genomic DNA sequence of α10 integrin contains 8
exons, and a Kozak sequence. The mouse genomic α10
sequence was used to generate a targeting vector for
knockout experiments.
15

Example 16
          Upregulation of α10 integrin subunit in chondrocytes
cultured in alginate beads
Material and methods
20   Human chondrocytes cultured in monolayer for 2 weeks
were detached with trypsin-EDTA and introduced into algi-
nate beads. Chondrocytes cultured in alginate are known
to preserve their phenotype while chondrocytes cultured
in monolayer are dedifferentiated. After 11 days chondro-
25   cytes cultured either in alginate or on monolayer were
isolated and surface labelled with [125]I. The α10 integrin
subunit was then immunoprecipitated with polyclonal
antibodies recognising the cytoplasmic domain of α10
(see Example 6 and Camper et al, JBC, 273, 20383-20389
30   (1998)).
Results
          As shown in figure 16 chondrocytes cultured in algi-
nate beads (lanes 3 and 4) upregulated their protein
expression of α10β1. This was in contrast to chondrocytes
35   cultured in monolayer (lanes 1 and 2) which had a very
low expression of α10β1. Immunoprecipitation with ab con-
trol antibody is shown in lanes 1 and 3.It is known that

chondrocytes preserve their cartilage specific matrixpro-
duction in alginate cultures but not in monolayer culture
which point to that alginate preserve the phenotype of
chondrocytes. These results support that α10 integrin

5   subunit can be used as a marker for differentiated chon-
drocytes.

Example 17
     Immunoprecipitation of the α10 integrin subunit from

10   human smooth muscle cells.
Material and methods
     Human smooth muscle cells were isolated from human
aorta. After one week in culture the cells were [125]I-
labelled, lysed and immunoprecipitated with antibodies

15   against the integrin subunit β1 (lane 1), α1 (lane 2), α2
(lane 3), α10 (lane 4), α3 (lane 5), control (lane 6)
(Figure 17). The experiment was done as described in
Example 7.
Results

20   The α10 antibody precipitated two bands from the
smooth muscle cells corresponding to the α10 and the β1
integrin subunit (Fig. 17).

Example 18

25   Construction of bacterial expression vector contain-
ing sequence for α10 splice region.
     A plasmid for intracellular expression in E. coli
of the alternatively spliced region (amino acid pos.
952-986, SEQ. ID 1) was constructed as described. The

30   alternatively spliced region were back-translated using
the E. coli high frequency codon table, creating a cDNA
sequence of 96% identity with the original sequence (SEQ.
ID 1 nucleotide pos 2940-3044). Using sequence overlap
extension (Horton et al., Biotechniques 8:528, 1990)

35   primer α10pfor (tab. I) and α10prev (tab. I) was used
to generate a double stranded fragment encoding the α10
amino acid sequence. This fragment was used as a PCR

template with primers α10pfor2 (tab. I) and α10prev2
(tab. I) in order to generate restriction enzyme site
for sub-cloning in a pET vector containing the Z-domain
of staphylococcal protein A, creating a fusion of the α10
spliced region with the amino terminal of the Z-domain
with trombin cleavage site residing in-between. The frag-
ment generated in the second PCR reaction is shown (SEQ
ID No. 3) also indicating the unique restriction enzymes
used for sub-cloning in the expression vector.

Table I

α10pfor	5'-GTTCAGAACCTGGGTTGCTACGTTGTTTCCGGTCTGATCATCTCCGCTCTGCTGCCGGCTGT-3'
α10pfor2	5'-GGGGGCATATGGTTCAGAACCTGGGTTGCTACGTTG-3'
α10prev	5'-GATAACCTGGGACAAGCTTAGGAAGTAGTTACCACCGTGAGCAACAGCCGGCAGCAGAGCGGA-3'
α10prev2	5'-GGGGGGATCCGCGCGGCACCAGGCCGCTGATAACCTGGGACAAGCTTAGGAAGT-3'

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